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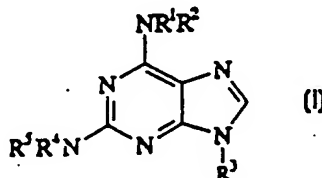
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(54) Title: PURINE INHIBITOR OF PROTEIN KINASES, G PROTEINS AND POLYMERASES



(57) Abstract

The present invention relates to purine analogs having general formula (I) or a pharmaceutically acceptable salt thereof, wherein R¹, R², R³, R⁴ and R⁵ are independently members selected from the group consisting of H, C₁-C₈ straight-chain, branched-chain, saturated and unsaturated alkyl, C₁-C₈ straight-chain, branched-chain, saturated and unsaturated substituted alkyl, aryl and substituted aryl, that inhibit, *inter alia*, protein kinases, G-proteins and polymerases. In addition, the present invention relates to methods of using such purine analogs to inhibit protein kinases, G-proteins, polymerases and other cellular processes and to treat cellular proliferative diseases.

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7, H-8, KN-62), sphingosine, tyrphostins and others, but in most cases these inhibitors display broad specificity. Only some pseudosubstrate autoinhibitory peptides show a high degree of specificity.

Cyclin-dependent kinases (CDK), in particular, have recently raised considerable interest in view of their essential role in the regulation of the cell division cycle (CDC) (*see*, Nigg, E.A. (1993), *Trends in Cell Biol.*, 3:296-301; and Sherr, C.S. (1993), *Cell*, 73:1059-1065). CDKs are highly conserved among eukaryotic species. Higher eukaryotic cells contain several isoforms of CDKs that become activated in specific phases of the cell cycle. CDKs consist of a catalytic subunit, the prototype of which is CDC2, and a regulatory subunit (cyclin). Six human CDK proteins have been described so far (*see*, Meyerson, M., *et al.* (1992), *EMBO J.*, 11:2909-2917; Meyerson, M., *et al.* (1994), *Mol. Cell. Biol.*, 14:2077-2086; and Van den Heuvel, S., *et al.* (1993), *Science*, 262:2050-2054), namely, CDK1 (also known as CDC2) and CDK2-6. With the exception of CDK3, for which the regulatory cyclin has not yet been identified, all these CDKs proteins are regulated by the transient association with one member of the cyclin family, *i.e.*, cyclin A (CDC2, CDK2), B1-B3 (CDC2), D1-D3 (CDK2, CDK4, CDK5, CDK6), E (CDK2). Each step of the cell cycle is thought to be regulated by such CDK complexes: G1/S transition (CDK2/cyclin E, CDK3/unknown cyclin, CDK4/cyclin D1-D3, CDK6/cyclin D3), S phase (CDK2/cyclin A), G2 (CDC2/cyclin A), G2/M transition (CDC2/cyclins B).

CDKs are able to phosphorylate many proteins that are involved in cell cycle events, including histones, lamins and tumor suppressor proteins, such as the retinoblastoma gene product pRb (*see*, Norbury, C., *et al.*, *supra*, Matsushime, H., *et al.* (1992), *Cell*, 71:323-334, Nigg, E.E. (1993), *Curr. Opin. Cell. Biol.*, 5:187-193). In accordance with their central role in the cell cycle, enzyme activity is tightly controlled by multiple mechanisms. Kinase activation requires complex formation with regulatory cyclin proteins as described above, followed by an activating phosphorylation on Thr-161 in CDC2 or the corresponding Thr in the other CDKs (*see*, *e.g.*, Gould, K.L., *et al.* (1991), *EMBO J.*, 10:3297-3309; Desai, D., *et al.* (1992), *Mol. Biol. Cell*, 3:571-582; Solomon, M.J., *et al.* (1992), *Mol. Biol. Cell*, 3:13-27). In addition, enzyme activity is negatively regulated by phosphorylations at Tyr-15 and/or Thr-14 (*see*, *e.g.*, Solomon, M.J., *et al.*, *supra*; Gu, Y., *et al.* (1992), *EMBO J.*, 11:3995-4005; Krek, W., *et al.* (1991), *EMBO J.*, 10:3331-3341; Norbury, C., *et al.* (1991), *EMBO J.*

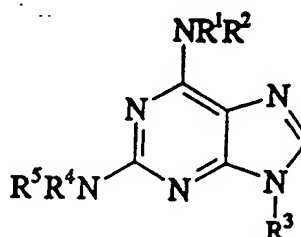
10:3321-3329; Parker, L.L., *et al.* (1992), *Proc. Natl. Acad. Sci. U.S.A.*, 89:2917-2921; McGowan, C.H., *et al.* (1993), *EMBO J.*, 12:75-85), or by complex formation with inhibitor proteins like p16 (*see*, Serrano, M., *et al.* (1993), *Nature (London)*, 366:704-707; Kamb, A., *et al.* (1994), *Nature (London)*, 264:436-440; Nobori, T., *et al.* (1994), *Nature (London)*, 368:753-756), p27 (*see*, Polyak, K., *et al.* (1994), *Cell*, 78:59-66; Toyoshima, H., *et al.* (1994), *Cell*, 78:67-74), p28 (*see*, Hengst, L., *et al.* (1994), *Proc. Natl. Acad. Sci. U.S.A.*, 91:5291-5295) and p21 (*see*, Gu, Y., *et al.* (1993), *Nature (London)*, 366:707-710; Xiiong, Y., *et al.* (1993), *Nature (London)*, 366:701-704; Harper, J.W., *et al.* (1993), *Cell*, 75:805-816; Dulic, V., *et al.* (1994), *Cell*, 76:1013-1023), the latter being inducible by p53. Especially noteworthy is the fact that deletions of the p16 gene were found in over 50% of all human malignant cell lines tested (*see*, Kamb, A., *supra*, Nobori, T., *et al.*, *supra*), although much less in primary tumor cells (*see*, Spruck III, C.H., *et al.* (1994), *Nature (London)*, 370:183-184), implicating p16 functions as tumor suppressor protein. Thus, both the cell growth signals transmitted through many oncogene products and the growth inhibitory signals from several tumor suppressor proteins modulate the activity of CDKs. Although mutations in CDKs themselves have not been associated with cancer, cyclin overexpression has been linked to tumorigenesis (*see*, Hunter, T., *et al.* (1991), *Cell*, 66:1071-1074; Keyomarsi, K., *et al.* (1993), *Proc. Natl. Acad. Sci. U.S.A.*, 90:1112-1116; Wang, T.C., *et al.* (1994), *Nature (London)*, 369:669-671.) Hence, CDKs are a promising target for developing inhibitors with antineoplastic effects and for the treatment of cell-proliferative diseases.

The purine ring system is a key structural element of the substrates and ligands of many biosynthetic, regulatory and signal transduction proteins including cellular kinases, G proteins and polymerases. As such, the purine ring system has been a good starting point in the search for inhibitors of many biomedically significant processes. In fact, while purine analogs were being screened for inhibition of various protein kinases, a relatively selective inhibitor, olomoucine (Figure 1), was identified that competitively inhibits CDK2/cyclin A with an IC_{50} of 7 μ M (*see*, Vesely, J., *et al.*, (1994) *Eur. J. Biochem.*, 224:771-786). Although olomoucine exhibits moderate inhibitory activity and good selectivity for the CDK/cyclin protein kinases, it would be advantageous to identify other purine analogs that have increased affinity and specificity for protein kinases as well as G proteins and polymerases. Quite surprisingly, the present invention provides such analogs.

SUMMARY OF THE INVENTION

The present invention provides (i) purine analogs that, *inter alia*, inhibit protein kinases, G proteins and polymerases; (ii) methods of using such purine analogs to inhibit protein kinases, G proteins, polymerases and other cellular processes; and (iii) pharmaceutical compositions comprising such purine analogs.

In one embodiment, the present invention provides purine analogs having the generally formula:



I

or a pharmaceutically acceptable salt thereof.

In Formula I, R¹, R², R⁴ and R⁵ are independently selected and are functional groups including, but not limited to, H, C₁-C₈ straight-chain, branched-chain, saturated and unsaturated alkyl, C₁-C₈ straight-chain, branched-chain, saturated and unsaturated substituted alkyl, aryl and substituted aryl.

In another embodiment, the present invention provides pharmaceutical compositions comprising the purine analog compounds of the invention and a pharmaceutically acceptable carrier.

In another embodiment, the present invention provides a method of inhibiting a protein selected from the group consisting of protein kinases, G proteins and polymerases, the method comprising contacting the protein with a purine analog of the present invention. In a preferred embodiment, the protein is a protein kinase. In an even more preferred embodiment, the protein kinase is a cyclin-dependent kinase. In an even more preferred embodiment, the cyclin-dependent kinase is a member selected from the group consisting of CDK1 (CDC2), CDK2, CDK3, CDK4, CDK5, CDK6, CDK7 and CDK8 and, in particular, CDK1 and CDK5.

In another embodiment, the present invention provides a method of treating a cellular proliferative disease, the method comprising administering to a mammal having the disease a therapeutically effective amount of a purine analog of the present invention.

5 In yet another embodiment, the present invention provides a method of inhibiting the growth of a tumor cell, the method comprising contacting the tumor cell with a purine analog of the present invention. In a preferred embodiment, the tumor cell is selected from the group consisting of lung, colon, breast, ovarian, prostate and hepatic cells. In a preferred embodiment, the tumor cell is in a mammalian subject. In another
10 preferred embodiment, the purine analog is formulated in a pharmaceutically acceptable form with an excipient or carrier and administered orally. In another embodiment, this method further comprising the step of observing for a reduction in the growth of a tumor cell.

15 In still another embodiment, the present invention provides a method of treating a neurodegenerative disease, the method comprising administering to a mammal having the disease a therapeutically effective amount of a purine analog of the present invention.

Other features, objects and advantages of the invention and its preferred embodiments will become apparent from the detailed description which follows.

20 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 sets forth the structure of olomoucine and the numbering scheme for the purine nucleus.

Figures 2 and 3 illustrate the IC_{50} for representative compounds from Table 1.

25 DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

30 The present invention provides (i) purine analogs that, *inter alia*, inhibit protein kinases, G proteins and polymerases; (ii) methods of using such purine analogs to inhibit protein kinases, G proteins, polymerases and other cellular processes; and (iii) pharmaceutical compositions comprising such purine analogs.

A. DEFINITIONS

The term "independently selected" is used herein to indicate that the R groups, *e.g.*, R¹, R², R⁴ and R⁵, can be identical or different (*e.g.*, R¹, R² and R³ may all be substituted alkyls or R¹ and R² may be a substituted alkyl and R³ may be an aryl, *etc.*).

A named R group will generally have the structure which is recognized in the art as corresponding to R groups having that name. For the purposes of illustration, representative R groups as enumerated above are defined herein. These definitions are intended to supplement and illustrate, not preclude, the definitions known to those of skill in the art.

The term "alkyl" is used herein to refer to a branched or unbranched, saturated or unsaturated, monovalent hydrocarbon radical having from 1-12 carbons and preferably, from 1-6 carbons. When the alkyl group has from 1-6 carbon atoms, it is referred to as a "lower alkyl." Suitable alkyl radicals include, for example, methyl, ethyl, n-propyl, i-propyl, 2-propenyl (or allyl), n-butyl, t-butyl, i-butyl (or 2-methylpropyl), *etc.*

"Substituted alkyl" refers to alkyl as just described including one or more functional groups such as lower alkyl, aryl, acyl, halogen (*i.e.*, alkylhalos, *e.g.*, CF₃), hydroxy, amino, alkoxy, alkylamino, acylamino, acyloxy, aryloxy, aryloxyalkyl, mercapto, both saturated and unsaturated cyclic hydrocarbons, heterocycles and the like. These groups may be attached to any carbon of the alkyl moiety.

The term "aryl" is used herein to refer to an aromatic substituent which may be a single aromatic ring or multiple aromatic rings which are fused together, linked covalently, or linked to a common group such as a methylene or ethylene moiety. The common linking group may also be a carbonyl as in benzophenone. The aromatic ring(s) may include phenyl, naphthyl, biphenyl, diphenylmethyl and benzophenone among others.

The term "arylalkyl" is used herein to refer to a subset of "aryl" in which the aryl group is attached through an alkyl group as defined herein.

"Substituted aryl" refers to aryl as just described including one or more functional groups such as lower alkyl, acyl, halogen, alkylhalos (*e.g.*, CF₃), hydroxy, amino, alkoxy, alkylamino, acylamino, acyloxy, mercapto and both saturated and unsaturated cyclic hydrocarbons which are fused to the aromatic ring(s), linked covalently or linked to a common group such as a methylene or ethylene moiety. The

linking group may also be a carbonyl such as in cyclohexyl phenyl ketone. The term "substituted aryl" encompasses "substituted arylalkyl."

"Substituted arylalkyl" defines a subset of "substituted aryl" wherein the substituted aryl group is attached to the nucleus by an alkyl group as defined herein.

5 The term "halogen" is used herein to refer to fluorine, bromine, chlorine and iodine atoms.

The term "hydroxy" is used herein to refer to the group —OH.

10 The term "amino" is used herein to refer to the group —NRR', where R and R' may independently be hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl or acyl.

15 The term "alkoxy" is used herein to refer to the —OR group, where R is a lower alkyl, substituted lower alkyl, aryl, substituted aryl, arylalkyl or substituted arylalkyl wherein the alkyl, aryl, substituted aryl, arylalkyl and substituted arylalkyl groups are as described herein. Suitable alkoxy radicals include, for example, methoxy, ethoxy, phenoxy, substituted phenoxy, benzyloxy, phenethyloxy, t-butoxy, *etc.*

The term "alkylamino" denotes secondary and tertiary amines wherein the alkyl groups may be either the same or different and may consist of straight or branched, saturated or unsaturated hydrocarbons.

20 The term "heterocyclic" is used herein to describe a monovalent group having a single ring or multiple condensed rings from 1-12 carbon atoms and from 1-4 heteroatoms selected from nitrogen, sulfur or oxygen within the ring. Such heterocycles are, for example, tetrahydrofuran, morpholine, piperidine, pyrrolidine, thiophene, pyridine, isoxazole, phthalimide, pyrazole, indole, furan, benzo-fused analogs of these rings, *etc.*

25 The term "substituted heterocyclic" as used herein describes a subset of "heterocyclic" wherein the heterocycle nucleus is substituted with one or more functional groups such as lower alkyl, acyl, halogen, alkylhalos (*e.g.*, CF₃), hydroxy, amino, alkoxy, alkylamino, acylamino, acyloxy, mercapto, *etc.*

30 The term "pharmaceutically acceptable salt" refers to those salts of compounds which retain the biological effectiveness and properties of the free bases and which are obtained by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. Pharmaceutically

acceptable salts include, for example, alkali metal salts, such as sodium and potassium, alkaline earth salts and ammonium salts.

The term "contacting" is used herein interchangeably with the following: combined with, added to, mixed with, passed over, incubated with, flowed over, *etc.*

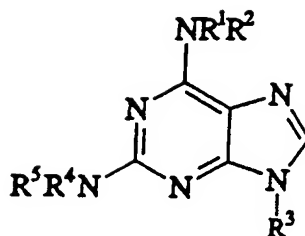
Moreover, the purine compounds of present invention can be "administered" by any conventional method such as, for example, parenteral, oral, topical and inhalation routes as described herein.

"An amount sufficient" or "an effective amount" is that amount of a given purine analog which exhibits the binding/inhibitory activity of interest or, which provides either a subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer.

B. PURINE ANALOGS

The purine ring is a key structural element of the substrates and ligands of many biosynthetic, regulatory and signal transduction proteins including cellular protein kinases, G proteins and polymerases. Quite importantly, the present invention provides purine analogs which can be used to inhibit such proteins and, thus, many biomedically important processes. More particularly, the present invention provides purine analogs that inhibit, *inter alia*, protein kinases, G proteins, polymerases and other cellular processes. As such, the purine analogs of the present invention can be used to block cell-cycle progression, cellular proliferation, apoptosis as well as other cellular processes. The purine analogs of the present invention are active in the subnanomolar and submicromolar ranges.

In one embodiment, the present invention provides purine analogs having the generally formula:



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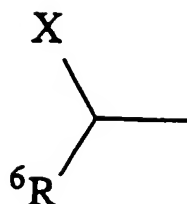
or a pharmaceutically acceptable salt thereof.

In Formula I, R^1 , R^2 , R^4 and R^5 are independently selected and are functional groups including, but not limited to, H, $\text{C}_1\text{-C}_8$ straight-chain, branched-chain, saturated and unsaturated alkyl, $\text{C}_1\text{-C}_8$ straight-chain, branched-chain, saturated and unsaturated substituted alkyl, aryl and substituted aryl.

Within the scope of the above Formula I, certain embodiments are preferred, namely those in which R^1 and R^2 are independently selected and are functional groups including, but not limited to, H, aryl, substituted aryl, $\text{C}_1\text{-C}_8$ straight-chain, saturated alkyl substituted with aryl and $\text{C}_1\text{-C}_8$ straight-chain, saturated alkyl substituted with substituted aryl; R^3 is a functional group including, but not limited to, $\text{C}_1\text{-C}_8$ branched-chain saturated alkyl and $\text{C}_1\text{-C}_8$ branched-chain unsaturated alkyl; and R^4 and R^5 are independently selected and are functional groups including, but not limited to, H, $\text{C}_1\text{-C}_8$ straight-chain, branched-chain, saturated and unsaturated alkyl, $\text{C}_1\text{-C}_8$ straight-chain, branched-chain, saturated and unsaturated substituted alkyl, aryl and substituted aryl.

In another preferred embodiment, R^1 and R^2 are independently selected and are functional groups including, but not limited to, H, unsubstituted aryl and substituted aryl; R^3 is isopropyl; and R^4 and R^5 are independently selected and are functional groups including, but not limited to, H, $\text{C}_1\text{-C}_8$ saturated and unsaturated branched-chain alkyl and $\text{C}_1\text{-C}_8$ saturated and unsaturated branched-chain substituted alkyl.

In another preferred embodiment, R^4 and R^5 are independently selected and are functional groups including, but not limited to, H, and



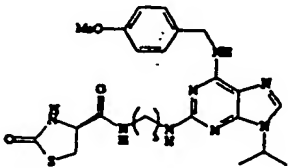
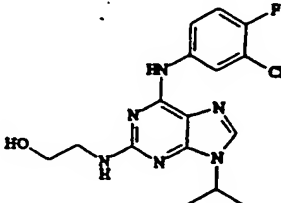
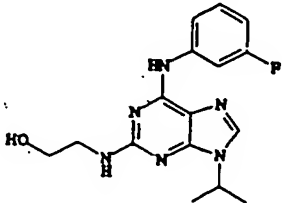
wherein X is a member selected from the group consisting of H, OH, CH₂OH, C(O)NH₂, SH, COOH or a pharmaceutically acceptable salt thereof and COOR⁷, wherein R⁷ is lower alkyl; and R⁶ is a member selected from the group consisting of H, C₁-C₈ straight-chain alkyl, C₁-C₈ branched-chain alkyl, C₁-C₈ straight-chain substituted alkyl, C₁-C₈ branched-chain substituted alkyl.

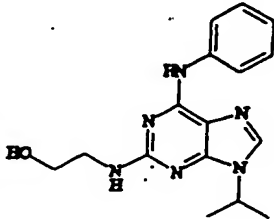
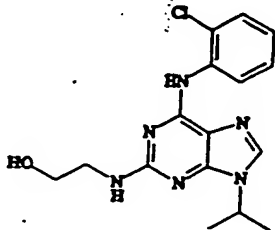
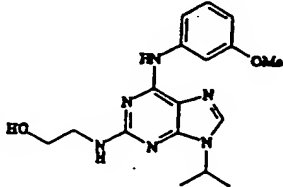
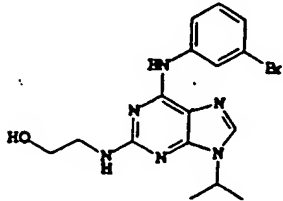
With respect to above embodiment, X is preferably COOH; and R⁶ is independently selected and is a functional group including, but not limited to, H, -CH₃, -(CH₂)₃NHC(=NH)NH₂, -CH₂CONH₂, -CH₂CO₂H, -CH₂SH, -(CH₂)₂CONH₂, -(CH₂)₂CO₂H, -CH₂(4-imidazolyl), -CH(CH₃)CH₂CH₂, -CH₂CH(CH₃)₂, -(CH₂)₄NH₂, -(CH₂)₂SCH₃, -CH₂Ph, -CH₂OH, -CH(CH₃)OH, -CH₂(3-indolyl), -CH₂(4-hydroxyphenyl) and -CH(CH₃)₂.

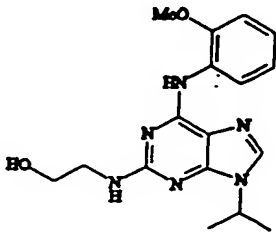
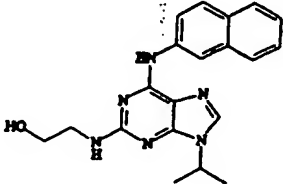
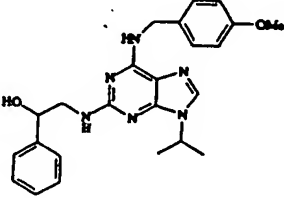
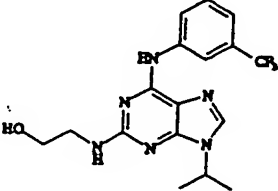
In such embodiments, R¹ and R² are independently selected and are functional groups including, but not limited to, H and aryl substituted in at least one of positions 3, 4, 5 with a member independently selected from the group consisting of halogen, alkoxy, trihalomethyl, amino, hydroxyl, thiol, sulfonic acid, sulfonic acid, amide, ester and carboxylic acid.

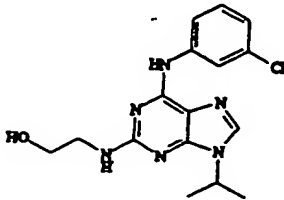
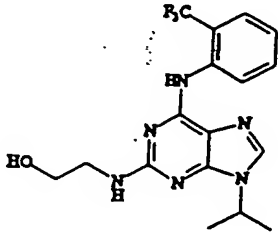
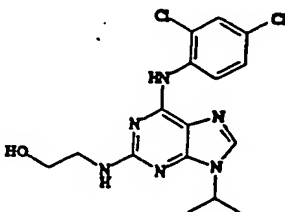
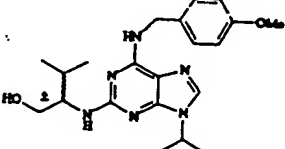
Table 1 sets forth purine compounds in accordance with the present invention which are particularly preferred. The compounds in this table and throughout this specification are referred to by code numbers, which are used for convenience only, and are strictly arbitrary for purposes of this invention.

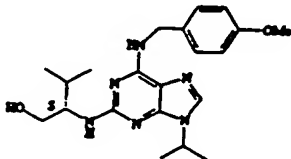
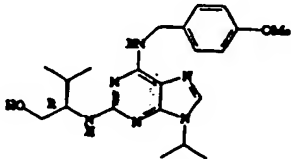
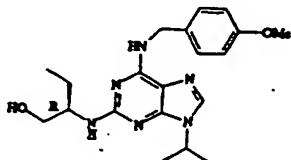
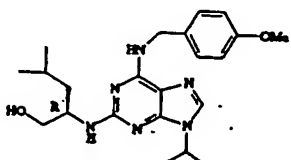
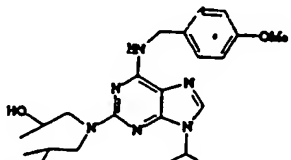
Table 1. Exemplary Purine Analogs

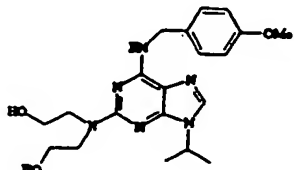
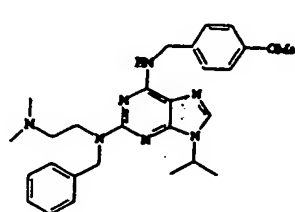
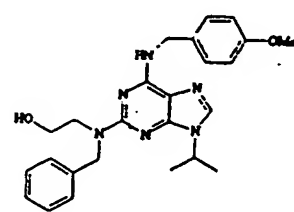
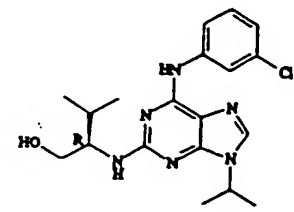
Structure	Code Name	IC ₅₀ cdc2/cyclinB (additional kinases)
Class 1		
	NG-30	330nM 2000nM(CDK2/cyclinE) > 33,000nM(GSK-3) 8,000nM(erk1)
Class 2a		
	NG-64	290nM
	NG-65	400nM

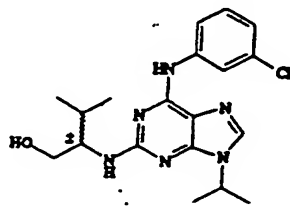
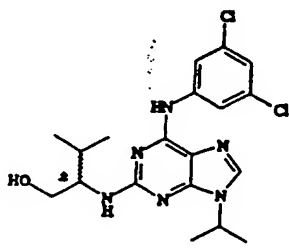
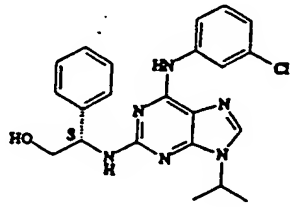
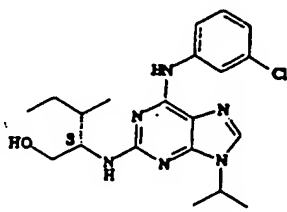
	NG-42	4300nM
	NG-43	4300nM
	NG-44	500nM
	NG-45	270nM

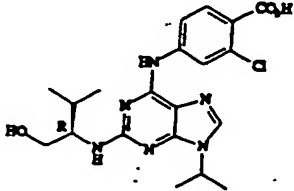
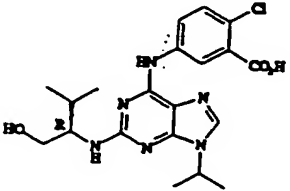
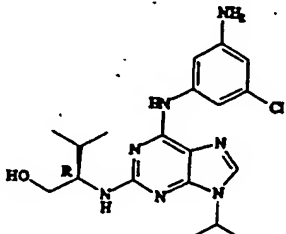
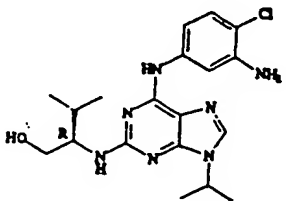
 <chem>CC(C)N1C=NC2=C1N=CN2C(=N2)NC(CO)CCN3C=NC=C(C=C3)OC</chem>	NG-46	9000nM
 <chem>CC(C)N1C=NC2=C1N=CN2C(=N2)NC(CO)CCN3C=CC=C4C=CC=CC=C4C3</chem>	NG-47	430nM
 <chem>CC(C)N1C=NC2=C1N=CN2C(=N2)NC(CO)CCN(Cc3ccccc3)C(=N2)NC4=CC=C(C=C4)OC</chem>	NG-50	2800nM
 <chem>CC(C)N1C=NC2=C1N=CN2C(=N2)NC(CO)CCN3C=CC=C(C=C3)Cl</chem>	NG-51	420nM

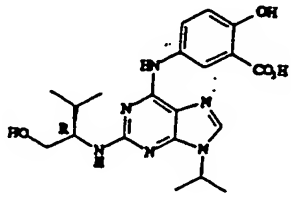
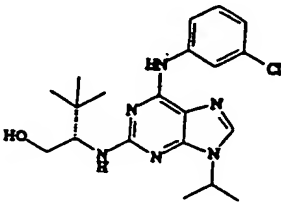
	NG-52	220nM
	NG-53	10,000nM
	NG-54	2700nM
Class 2b		
	NG-35	150nM 140(cdk2/cylinE) 15nM(cdk5/p25) 4500(GSK-3) 3000(erk1)

	NG-76	600nM 400(CDK2/cyclinE)
	NG-75	230nM 150(CDK2/cyclinE)
	NG-33	130nM 80nM(CDK2/cyclinE) 20,000nM(GSK-3) > 10,000nM(erk1)
	NG-36	100nM 100nM(cd2/cyclinE) 13,000nM(GSK-3) > 10,000nM(erk1)
Class 2c		
	NG-16	240nM 180nM(CDK2/cyclinE) 23,000nM(GSK-3) 50,000nM(erk1)

	NG-26	330nM 230nM(CDK2/cyclinE) > 33,000nM(GSK-3) 33,000nM(erk1)
	NG-40	600nM
	NG-49	2800nM
Class 3		
	NG-60	35nM 30nM (CDK2/CyclinE)

	NG-56	35nM 55nM (CDK2/CyclinE)
	NG-57	400nM
	NG-59	800nM
	NG-62	500nM

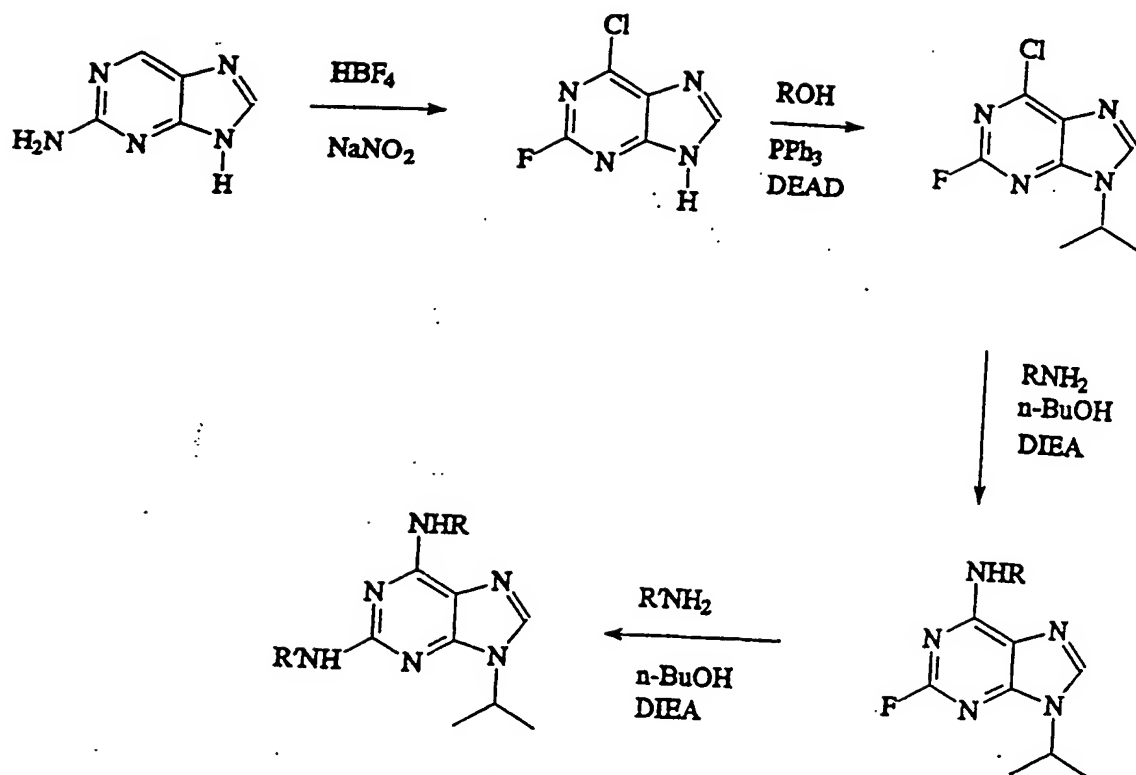
	NG-95	approx. 20nM
	NG-96	approx. 30nM
	NG-97	30nM
	NG-98	30nM

	NG-94	approx. 100nM
	NG-61	2300nM

^a These IC₅₀s can be compared with other known small molecule inhibitors of CDK2 (see, Figures 2 and 3).

It will be readily appreciated by those of skill in the art that depending on the substituents, the purine analogs of the present invention can be a racemic mixture or either of a pair of diastereomers or enantiomers.

The purine analogs of the present invention can be synthesized in a variety of ways, using conventional synthetic chemistry techniques. Typically, the compounds of the present invention are prepared according to the reaction scheme set forth in Scheme I, wherein R¹, R², R³, R⁴, and R⁵ are as defined above. The use of appropriate organic solvents, temperature and time conditions for running the reactions are within the level of skill in the art. Reactions of this type are generally described by Norman, *et al.*, *J. Am. Chem. Soc.* 118:7430-7431 (1996); and Gray, *et al.*, *Tetrahedron Letters*, 38:1161-1164 (1997), the teachings of which are incorporated herein by reference. Moreover, suitable synthesis reactions are illustrated herein by the representative examples. Necessary starting materials can be obtained by standard procedures of organic chemistry.



SCHEME 1

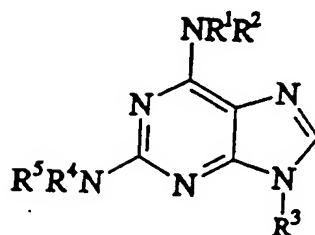
Briefly, as illustrated in Scheme I, a purine derivative with a halogen at the 2-position is alkylated at the 9-position with an alcohol using the Mitsunobu alkylation. Following the alkylation, the purine derivative is aminated at the 6-position with an amine. Once prepared, the purine analogs can be purified (*e.g.*, by TLC), characterized (*e.g.*, by Reverse Phase HPLC) and analyzed (*e.g.*, by high resolution spectroscopy using, for example, ¹H NMR or FAB-MS).

C. USES FOR THE PURINE ANALOGS OF THE PRESENT INVENTION

The purine analogs of the present invention can be used either *in vitro* or *in vivo* for a variety of purposes. As mentioned, the purine analogs of the present invention can be used to inhibit protein kinases, G proteins and polymerases. Moreover, the purine analogs of the present invention can be used to treat cellular-proliferative diseases. In addition, the compounds of the present invention can be used as molecular tools and molecular probes.

As such, in one embodiment, the present invention provides a method of inhibiting a protein kinase, a G protein or a polymerase, the method comprising

contacting the protein kinase, the G protein or the polymerase with a purine analog having the general formula:



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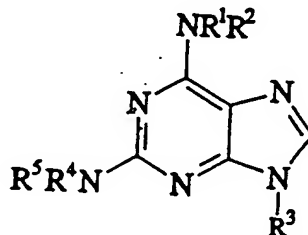
or a pharmaceutically salt thereof. The prior discussions pertaining to R¹, R², R⁴, R⁵ and R⁶, their definitions and preferred embodiments are fully applicable to the purine analogs used in this method and, thus, will not be repeated. The protein kinase, G protein or polymerase is contacted with the purine analog in an amount sufficient to effect inhibition.

Protein kinases which can be inhibited using the purine analogs of the present invention include, but are not limited to, cyclin-dependent kinases (CDKs), MAP kinases (p38, ERK), (MAPK/MEK/MEKK), cAMP - dependent kinase, c-GMP - dependent kinase, Calmodulin - dependent kinase, CSK (C-src like kinase) pp 60 c-src, myosin light chain kinase, JNK kinase, IKB kinase, Protein kinase C, *etc.* In a presently preferred embodiment, the protein kinase is a CDK. Such CDKs include CDK1 (or, interchangeably, CDC2) and CDK2-CDK8. In an even more preferred embodiment, the CDK is CDC2, CDK2 or CDK5 as many of the purine analogs of the present invention exhibit increased affinity and specificity with respect to these CDKs. G proteins can be inhibited using the compounds of the present invention include, but are not limited to, GTP binding proteins. Polymerases which can be inhibited using the purine analogs of the present invention include, but are not limited to, DNA polymerase α , DNA polymerase σ , DNA topoisomerase I, topoisomerase II, phosphatases, telomerases, *etc.* Other protein kinases, G proteins and polymerases which can be inhibited using the purine analogs of the present invention will be known to those of skill in the art.

Purine analogs of the present invention suitable for use in inhibiting protein kinases, G proteins or polymerase can readily be identified using *in vitro* and *in*

vivo screening assays. For instance, purine analogs having protein kinase inhibitory activity can be screened for using the CDK2/CYCLIN A microtiter-based solution-phase protein kinase assay described in the examples and by Buxbaum, J.D., *et al. Anal. Biochem.* 1988, 169:209-215. Similarly, numerous assays exist which can be used to screen a given purine analog for G protein inhibitory activity or polymerase inhibitory activity. Such assays are described, for example, by Vesley, J., *et al., Eur. J. Biochem.*, 1994, 224:771-786, the teachings of which are incorporated herein by reference. Other assays known to and used by those of skill in the art can also be used to screen a given purine analog for inhibitory properties against protein kinases, G proteins and polymerases.

In addition, the purine analogs of the present invention can be used to treat cellular-proliferative disease, the method comprising administering to a mammalian subject having a cellular-proliferative disease a therapeutically effective amount of a purine analog having the general formula:

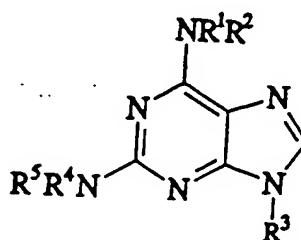


or a pharmaceutically salt thereof. The prior discussions pertaining to R¹, R², R⁴, R⁵ and R⁶, their definitions and preferred embodiments are fully applicable to the purine analogs used in this method and, thus, will not be repeated.

Cellular proliferative diseases which can be treated using the purine analogs of the present invention include, but are not limited to, abnormal stimulation of endothelial cells (*e.g.*, atherosclerosis), solid tumors and tumor metastasis, benign tumors, for example, hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, vascular malfunctions, abnormal wound healing, inflammatory and immune disorders, Bechet's disease, gout or gouty arthritis, abnormal angiogenesis accompanying, for example, rheumatoid arthritis, psoriasis, diabetic retinopathy, other

ocular angiogenic diseases such as retinopathy of prematurity (retrolental fibroplastic), macular degeneration, corneal graft rejection, neurovascular glaucoma and Oster Webber syndrome, psoriasis, restinosis, fungal, parasitic and viral infections such cytomegaloviral infections.

5 In a preferred embodiment, the present invention provides a method of inhibiting the growth of a tumor cell, the method comprising contacting the tumor cell with a compound having the general formula:

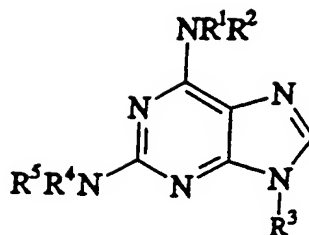


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10 or a pharmaceutically acceptable salt thereof. The prior discussions pertaining to R¹, R², R⁴, R⁵ and R⁶, their definitions and preferred embodiments are fully applicable to the purine analogs used in this method and, thus, will not be repeated.

15 In accordance with the above method, tumor cells include, but are not limited to, lung, colon, breast, ovarian, prostate and hepatic tumor cells as well as squamous cell carcinomas. In a presently preferred embodiment, the tumor cells are present in a mammalian subject. Mammalian subjects include, but are not limited to, humans, laboratory animals, domestic pets and farm animals. In a further preferred embodiment, the above method further comprises the step of observing for a reduction in the growth of the tumor cells.

20 In another embodiment, the present invention provides a method of treating cancer, the method comprising administering to a mammalian subject having cancer a therapeutically effective amount of a compound having the general formula:



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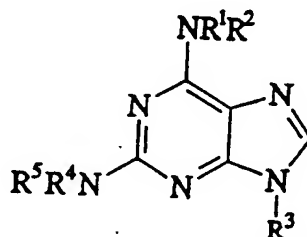
or a pharmaceutically acceptable salt thereof. The prior discussions pertaining to R^1 , R^2 , R^4 , R^5 and R^6 , their definitions and preferred embodiments are fully applicable to the purine analogs used in this method and, thus, will not be repeated.

5 The compounds of the present invention are useful for treating a wide variety of cancers. Such cancers include, by way of example and not limitation, carcinomas such as pharynx, colon, rectal, pancreatic, stomach, liver, lung, breast, skin, prostate, ovary, cervical, uterine and bladder cancers; leukemias; lymphomas; gliomas; retinoblastomas; and sarcomas. Moreover, in accordance with the above method, mammalian subjects include, but are not limited to, humans, laboratory animals, domestic pets and farm animals.

10 Purine analogs suitable for use in the methods of the present invention can readily be identified using *in vitro* and *in vivo* screening assays. Such assays may screen for the ability of a particular compound to inhibit malignant tumor cell growth or to abolish tumorigenicity of malignant cells *in vitro* or *in vivo*. For instance, tumor cell lines can be exposed to varying concentrations of a purine analog of interest, and the viability of the cells can be measured at set time points using the alamar Blue® assay (commercially available from BioSource, International of Camarillo, California). When alamar Blue dye is added to the culture medium, the dye is reduced by cellular
15 mitochondrial enzymes yielding a soluble product with substantially enhanced fluorescence. This fluorescence can be measured with a fluorimeter, whereby the signal is directly proportional to the cell number. Using this information, IC_{50} (concentration of compound lethal to 50% of a cell culture as compared to a control culture) values for the compounds of interest can be readily be calculated.

As will be appreciated by the skilled artisan, many varieties of malignant tumor cell cultures and cell lines can be used to screen for activity, including but not limited to MDA MB-231 (breast), MCF-7 (breast), MDA MB 468 (breast), Siha (squamous cell carcinoma), A549 (non-small cell lung), HL-60 (leukemia) Ovarcar-3 (ovarian), etc. In addition, the purine analogs of the present invention can be screened pm the National Cancer Institute panel of 60 human tumor cell lines. Of course, other *in vitro* and/or *in vivo* assays to screen for anti-tumor and/or anti-cancer activity known to and used by the skilled artisan can also be employed to identify effective purine analogs useful in the methods of the present invention.

In another preferred embodiment, the purine analogs of the present invention can be used to treat a neurodegenerative disease, the method comprising administering to a mammal having such a disease a therapeutically effective amount of a compound having the general formula:



or a pharmaceutically acceptable salt thereof. The prior discussions pertaining to R¹, R², R⁴, R⁵ and R⁶, their definitions and preferred embodiments are fully applicable to the purine analogs used in this method and, thus, will not be repeated.

Neurodegenerative diseases which can be treated using the purine analog compounds of the present invention include, but are not limited to, neurodegenerative pathologies involving multiple neuronal systems and/or brainstem including Alzheimer's disease, AIDS-related dementia, Leigh's disease, diffuse Lewy body disease, epilepsy, Multiple system atrophy, Guillain-Barre syndrome, lysosomal storage disorders such as lipofuscinosis, late-degenerative stages of Down's syndrome, Alper's disease, vertigo as result of CNS degeneration, etc. Other neurodegenerative diseases which can be treated

using the purine analogs of the present invention will be readily apparent to those of skill in the art.

In addition, in view of their cell-cycle arresting activities, the purine analogs of the present invention can be used to inhibit undesirable proliferation, including, as described above, cancer, psoriasis, growth of fungi, parasites, viruses, plants, *etc.* Moreover, the purine analogs of the present invention have apoptosis-inducing effects in actively dividing cells and, thus, this property can be advantageously used to treat various disease states associated with undesirable proliferation. Such uses are described, for example, in Meijer, L., *Trends in Cell Biology* (1986), 6:393-397, the teachings of which are incorporated herein by reference for all purposes.

In addition to the foregoing, the purine analogs of the present invention can be used *in vitro* as molecular tools and probes. For instance, since CDK inhibitors arrest cells both in G1 and late G2/early prophase, they can be used to synchronize cells when used preferably in combination with another synchronizing agent/method (*e.g.*, when used in combination with aphidicolin). Moreover, the purine analogs of In addition, immobilized CDK inhibitors can be used for affinity purification/depletion of CDKs from cellular extracts. Such purine analogs will be particularly useful for massive purification of expressed CDKs (for crystallography or screening purposes). In addition, such purine analogs are useful for comparative analysis of CDKs extracted from cells at difference developmental or cell-cycle stages (variation of concentration, kinase activity, post-translational modifications, *etc.*).

D. PHARMACEUTICAL FORMULATIONS/ROUTES OF ADMINISTRATION

The compounds, *i.e.*, purine analogs, of the present invention can be administered to a mammal, *e.g.*, a human patient, alone, in the form of a pharmaceutically acceptable salt, or in the form of a pharmaceutical composition where the compound is mixed with suitable carriers or excipient(s) in a therapeutically effective amount, *e.g.*, at doses effective to inhibit a protein kinase or a cellular process or achieve amelioration of symptoms of a disease associated with a protein kinase.

The compounds of this invention can be incorporated into a variety of formulations for therapeutic administration. More particularly, the compounds of the present invention can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated

into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, pills, powders, granules, dragees, gels, slurries, ointments, solutions, suppositories, injections, inhalants and aerosols. As such, administration of the compounds can be achieved in various ways, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, transdermal, intracheal, *etc.*, administration. Moreover, the compound can be administered in a local rather than systemic manner, for example via injection of the compound directly into a solid tumor, often in a depot or sustained release formulation. In addition, the compounds can be administered in a targeted drug delivery system, for example, in a liposome coated with tumor-specific antibody. Such liposomes will be targeted to and taken up selectively by the tumor.

The purine analogs of the present invention can be administered alone, in combination with each other, or they can be used in combination with other known compounds (*e.g.*, other drugs, such as anti-cancer drugs (*e.g.*, AZT), anti-mitotics, anti-inflammatories, antibiotics, corticosteroids, vitamins, *etc.*). More particularly, the compound of the present invention can be used in conjunctive therapy with other known chemotherapeutic or antineoplastic agents (*e.g.*, vinca alkaloids, antibiotics, antimetabolites, platinum coordination complexes, *etc.*). For instance, the compounds of the present invention can be used in conjunctive therapy with a vinca alkaloid compound, such as vinblastine, vincristine, taxol, *etc.*; an antibiotic, such as adriamycin (doxorubicin), dactinomycin (actinomycin D), daunorubicin (daunomycin, rubidomycin), bleomycin, plicamycin (mithramycin) and mitomycin (mitomycin C), *etc.*; an antimetabolite, such as methotrexate, cytarabine (AraC), azauridine, azaribine, fluorodeoxyuridine, deoxycoformycin, mercaptopurine, *etc.*; or a platinum coordination complex, such as cisplatin (cis-DDP), carboplatin, *etc.* In addition, those of skill in the art will appreciate that the compounds of the present invention can be used in conjunctive therapy with other known chemotherapeutic or antineoplastic compounds. In pharmaceutical dosage forms, the compounds may be administered in the form of their pharmaceutically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination with other pharmaceutically active compounds.

Suitable formulations for use in the present invention are found in *Remington's Pharmaceutical Sciences* (Mack Publishing Company, Philadelphia, PA, 17th ed. (1985)), which is incorporated herein by reference. Moreover, for a brief review of methods for drug delivery, *see*, Langer, *Science* 249:1527-1533 (1990), which

is incorporated herein by reference. The pharmaceutical compositions described herein can be manufactured in a manner that is known to those of skill in the art, *i.e.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. The following methods and
5 excipients are merely exemplary and are in no way limiting.

For injection, the compounds can be formulated into preparations by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher
10 aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives. Preferably, the compounds of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such
15 penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining with pharmaceutically acceptable carriers that are well known in the art. Such carriers enable the compounds to be formulated as tablets, pills, dragees, capsules, emulsions, lipophilic and hydrophilic suspensions, liquids, gels, syrups, slurries,
20 suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by mixing the compounds with a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose,
25 mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium
30 alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide,

lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

5 Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

 For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

15 For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas, or from propellant-free, dry-powder inhalers. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

25 The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules or in multidose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

30 Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic

fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter, carbowaxes, polyethylene glycols or other glycerides, all of which melt at body temperature, yet are solidified at room temperature.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various types of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in a therapeutically

effective amount. The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician. Determination of an effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any compound used in the method of the invention, a therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (*i.e.*, the concentration of test compound that is lethal to 50% of a cell culture), or the IC_{100} as determined in cell culture (*i.e.*, the concentration of compound that is lethal to 100% of a cell culture). Such information can be used to more accurately determine useful doses in humans. Initial dosages can also be estimated from *in vitro* or *in vivo* data.

Initial dosages can also be formulated by comparing the effectiveness of the compounds described herein in cell culture assays with the effectiveness of known drugs. For instance, when use as anticancer agents, initial dosages can be formulated by comparing the effectiveness of the compounds described herein in cell culture assays with the effectiveness of known anticancer drugs such as vincristine. In this method, an initial dosage can be obtained by multiplying the ratio of effective concentrations obtained in cell culture assay for the a compound of the present invention and a known anti-cancer drug by the effective dosage of the known anti-cancer drug. For example, if a compound of the present invention is twice as effective in cell culture assay than vincristine (*i.e.*, the IC_{50} of that compound is equal to one-half the IC_{50} of vincristine in the same assay), an initial effective dosage of the compound of the present invention would be one-half the known dosage for vincristine. Using these initial guidelines one having ordinary skill in the art could determine an effective dosage in humans.

Moreover, toxicity and therapeutic efficacy of the compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, by determining the LD_{50} , (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index and can be expressed as the ratio between LD_{50} and ED_{50} . Compounds which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and

animal studies can be used in formulating a dosage range that is not toxic for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See, e.g., Fingl *et al.*, 1975, In: *The Pharmacological Basis of Therapeutics*, Ch. 1, p. 1).

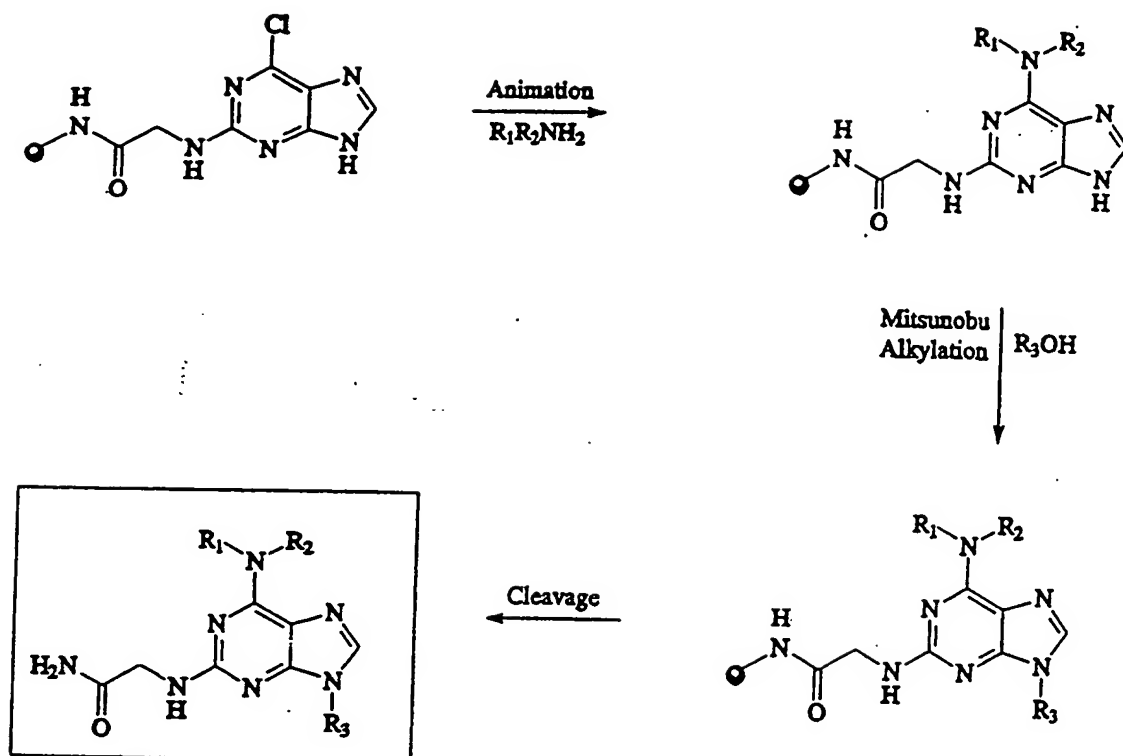
Dosage amount and interval may be adjusted individually to provide plasma levels of the active compound which are sufficient to maintain therapeutic effect. Usual patient dosages for oral administration range from about 50-2000 mg/kg/day, commonly from about 100-1000 mg/kg/day, preferably from about 150-700 mg/kg/day and most preferably from about 250-500 mg/kg/day. Preferably, therapeutically effective serum levels will be achieved by administering multiple doses each day. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration. One having skill in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

EXAMPLES

Example 1 illustrates a general synthetic scheme for producing the purine derivatives of the invention on a solid support. The solid-phase synthesis strategy exemplified by Scheme 2 involves attaching the growing compound to the solid-support via the side-chain at position 2 of the purine ring structure.

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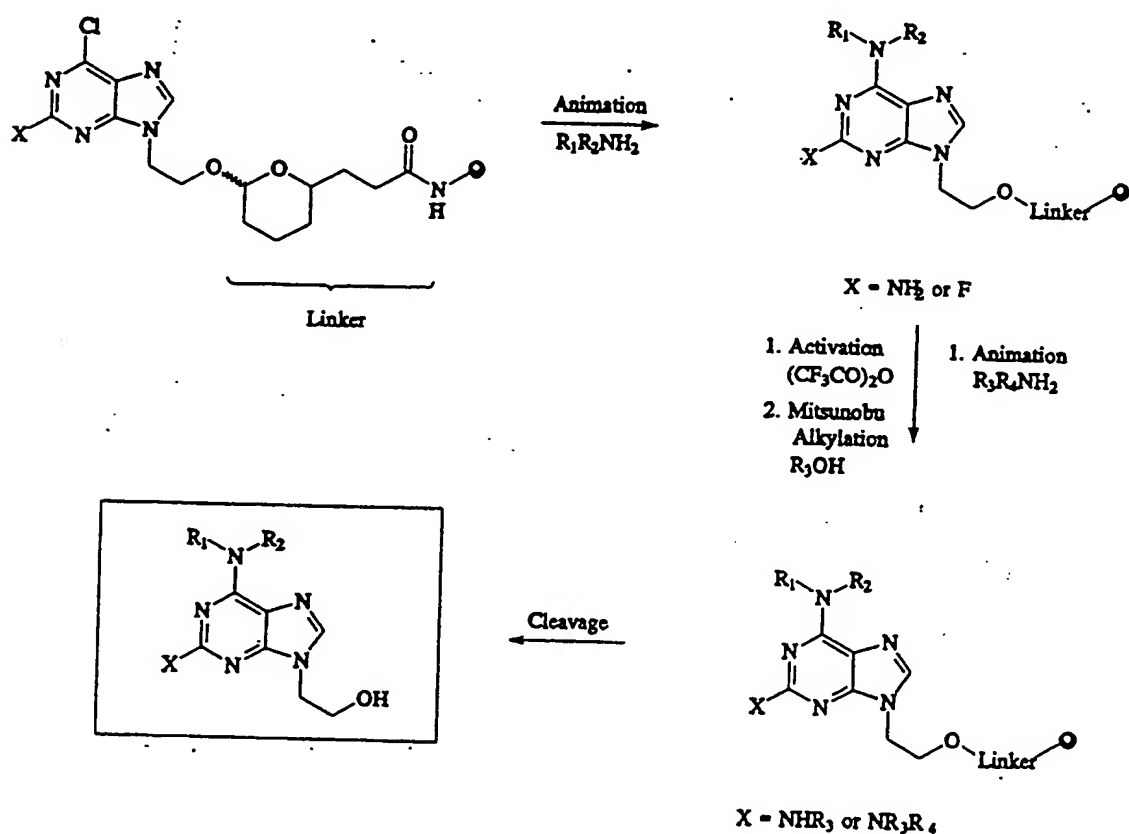


Scheme 1

EXAMPLE 2

Example 2 illustrates a generalized synthetic route to purine derivatives on a solid support. The solid-phase synthesis strategy exemplified by Scheme 2 involves attaching the growing compound to the solid-support via the side-chain at position 9 of the purine ring structure.

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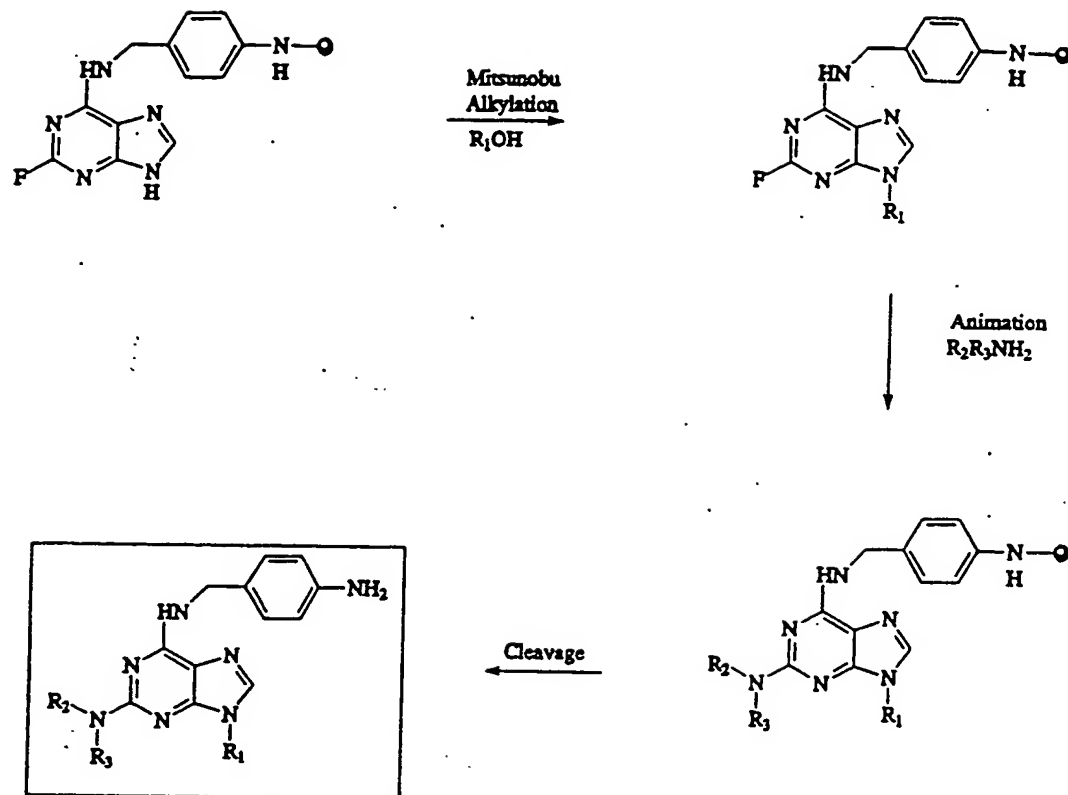


Scheme 2

EXAMPLE 3

Example 3 illustrates a general route to purine derivatives synthesized on a solid support. The route exemplified by Scheme 3 involves attaching the growing compound to the solid-support via the substituent at the 6-position of the purine ring.

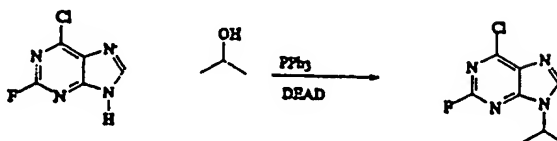
Library #3:



Scheme 3

EXAMPLE 4

Example 4 details the alkylation of position 9 of a purine nucleus. The synthetic route is summarized in Scheme 4.



Scheme 4

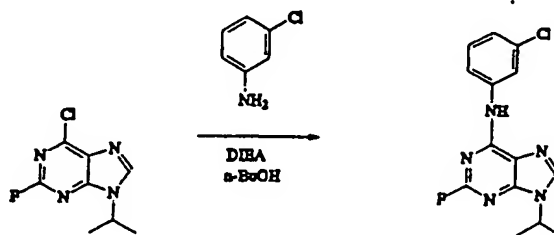
2-Fluoro-6-chloropurine (900 mg 5.20 mmol) and PPh₃ (3.0 g, 10.4 mmol) were combined in a flame-dried flask under N₂. Freshly distilled THF (60 mL) was added followed by 2-propanol (800 μ L, 10.4 mmol). The mixture was cooled to -10°C in an ethylene glycol/dry ice bath. DEAD (850 μ L, 10.4 mmol) was added over

10 min. The mixture was stirred at -10°C and gradually returned to room temperature over 3 hours.

The reaction was quenched by adding water (500 μL) to the reaction mixture. The solvent was removed in vacuo to viscous yellow oil. The oil was azeotroped with CH_2Cl_2 (2 x 10 mL) to remove trace THF. Purification was effected by column chromatography on silica gel eluted with CH_2Cl_2 . The CH_2Cl_2 was removed from the desired fraction. The desired product was isolated in 57% yield as a white powder.

EXAMPLE 5

Example 5 illustrates the synthetic route to amination of the

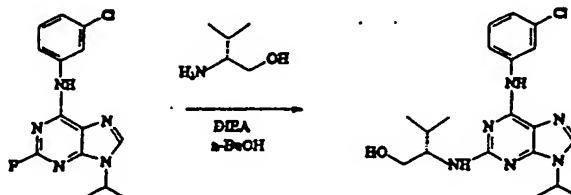


Scheme 5

The compound from Example 4 (3.75 g, 17.47 mmol) was combined with 3-chloroaniline (1.85 mL, 17.47 mmol) and diisopropylethylamine (3.05 mL, 17.47 mmol) in n-BuOH. The reaction mixture was heated to 70°C - 80°C for 11 hours. The n-BuOH was removed under vacuum and the resulting residue was suspended in H_2O to produce a slurry. The product was isolated by filtration, washed with small portions of CH_2Cl_2 and Et_2O . The product was dried first under a stream of air and then under vacuum. The desired product was isolated in 58% yield.

EXAMPLE 6

Example 6 details the amination of the 2-position of the purine ring system. The synthetic route is illustrated in Scheme 6.



Scheme 6

The compound from Example 5 (1.55 g, 5.10 mmol), 2-amino-3-methyl-1-butanol (559 μ L, 5.10 mmol) and diisopropylethylamine (892 μ L, 5.10 mmol) were combined in n-BuOH. The mixture was heated to approximately 100°C. The solvent was removed under reduced pressure and the residue purified by silica gel chromatography using 99:1 CH₂Cl₂:MeOH. The desired product was isolated in 71% yield.

The above examples illustrate both general and specific methods for synthesizing a wide array of the purine derivatives of the present invention.

EXAMPLE 7

This example illustrates a CDK2/CYCLIN A Microtiter Protein Kinase Assay which can be used to screen the purine analogs of the present invention for inhibitory activity.

1. Required buffers and solutions**a. Buffer A: 80 mM Tris (pH=7.2) mM MgCl₂**

Recipe: 4.84 g Tris (F.W. = 121.1 g/mol) 4.07 g MgCl₂ (F.W. = 203.31 g/mol) dissolved in 500 mL of ddH₂O. pH adjusted to 7.2 with HCl.

- b. **Histone H1 solution:** 0.45 mg/ml Histone H1 in 20 mM HEPES pH = 7.2.

Recipe: 5 mg of Histone H1 in 11.111 mL of 20 mM HEPES pH = 7.2. 477 mg of HEPES PROVIDED IN 1 mL ALIQUOTS. Store at -80°C.

- c. **ATP solution:** 90 μ M ATP, 300 μ g/mL BSA, 3 mM DTT.

Recipe: 9.25 mg DTT, 1.01 mg ATP (F.W. = 560 g/mol), 6 mg BSA dissolved in 20 mL ddH₂O. PROVIDED IN 1 mL ALIQUOTS. Store at -80°C.

- d. **CDK2 solution:** 10 mM HEPES pH = 7.2, 25 mM NaCl, 0.5 mM DTT, 10% glycerol. PROVIDED IN 192 μ L ALIQUOTS. Store at -80°C.

2. Stepwise Description of Assay.

- a. Prepare solutions of inhibitors at three times the desired final assay concentration in ddH₂O 15% DMSO by volume.

- b. Dispense 20 μ L of inhibitors to the well of a microtiter-formatted assay tray.

- c. Thaw Histone H1 solution (1 mL aliquot), ATP solution (1 mL aliquot) and CDK2 solution (192 μ L aliquot).

- d. Dilute 192 μ L of CDK2 solution into 2.1 mL of buffer A. Swirl to mix. Dispense 20 μ L of this solution to each well using a multichannel pipetman. (Note it is important to have a fairly pointed trough for loading the multichannel to avoid running out of solution.)

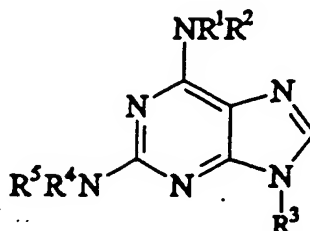
- e. Mix 1 mL of Histone H1 solution with 1 mL of the ATP solution in a 10 mL screw cap tube. Swirl to mix. Add 2-3 μL (Depending on how fresh your hot ATP is) of $\gamma\text{-}^{32}\text{P}$ -ATP (10 $\mu\text{Ci/mL}$). Mix thoroughly to get even distribution of hot ATP but watch out for BSA frothing! Dispense to wells with multichannel pipetman; mix the solution in the wells half a dozen times with the multichannel pipetman.
- f. Let reactions proceed for 30 minutes. While reactions are running you can
- i) Presoak a 9 x 12 cm piece of nitrocellulose (0.22 Micron) in water for 10 minutes.
 - ii) Load the nitrocellulose paper onto the dot blot. Screw the four clamps down (do this in a diagonal fashion) till they are finger tight. Then apply a vacuum and continue to tighten the screws until they are tight. Turn off the vacuum and load 100 μL of water into each well of the dot blot to rehydrate the membrane. Apply a weak vacuum to remove the excess water, but be careful not to dry out the membrane.
 - iii) Just before the 30 minutes is up add 35 μL of 10% TCA to each well of the dot blot.
- g. Using the multichannel pipetman transfer 35 μL of the reaction mixtures to each well of the dot blot in the same fashion as the ATP was dispensed (to insure equal reaction times).
- h. Add an additional 35 μL of 10% TCA and apply a weak vacuum until the wells are free of liquid. Repeat the process of adding 35 μL of 10% TCA and draining with the vacuum two more times. Remember to turn off vacuum when the wells are free of liquid or when you are refilling.

- i. Add 35 μ L of water to each well of the dot blot and apply a weak vacuum until the wells are free of liquid. Carry out this process a total of four times.
- j. Transfer the nitrocellulose membrane from the dot blot apparatus into a small tray containing enough water to cover the membrane. Let the membrane sit in the water for ten minutes then decant. Wash the membrane in this fashion with three batches of water.
- k. Let the membrane dry completely before analysis with the phosphoimager.

It is to be understood that the above description is intended to be illustrative and not restrictive. Many embodiments will be apparent to those of skill in the art upon reading the above description. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. The disclosures of all articles and references, including patent applications and publications, are incorporated herein by reference for all purpose.

WHAT IS CLAIMED IS:

1. A compound having the formula



wherein,

R^1 , R^2 , R^3 , R^4 and R^5 are independently members selected from the group consisting of H, $\text{C}_1\text{-C}_8$ straight-chain, branched-chain, saturated and unsaturated alkyl, $\text{C}_1\text{-C}_8$ straight-chain, branched-chain, saturated and unsaturated substituted alkyl, aryl and substituted aryl.

2. A compound according to claim 1, wherein

R^1 and R^2 are independently members selected from the group consisting of H, aryl, substituted aryl, $\text{C}_1\text{-C}_8$ straight-chain, saturated alkyl substituted with aryl and $\text{C}_1\text{-C}_8$ straight-chain, saturated alkyl substituted with substituted aryl;

R^3 is a member selected from the group consisting of $\text{C}_1\text{-C}_8$ branched-chain saturated alkyl and $\text{C}_1\text{-C}_8$ branched-chain unsaturated alkyl; and

R^4 and R^5 are independently members selected from the group consisting of H, $\text{C}_1\text{-C}_8$ straight-chain, branched-chain, saturated and unsaturated alkyl, $\text{C}_1\text{-C}_8$ straight-chain, branched-chain, saturated and unsaturated substituted alkyl, aryl and substituted aryl.

3. A compound according to claim 2, wherein

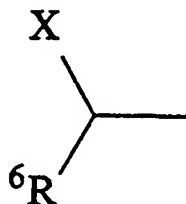
R^1 and R^2 are independently members selected from H, unsubstituted aryl and substituted aryl;

R^3 is isopropyl; and

R^4 and R^5 are independently members selected from the group consisting of H, C_1 - C_8 saturated and unsaturated branched-chain alkyl and C_1 - C_8 saturated and unsaturated branched-chain substituted alkyl.

4. A compound according to claim 3, wherein

R^4 and R^5 are independently members selected from the group consisting of H, and



wherein

X is a member selected from the group consisting of H, OH, CH_2OH , $C(O)NH_2$, SH, COOH or a pharmaceutically acceptable salt thereof and $COOR^7$, wherein R^7 is lower alkyl; and

R^6 is a member selected from the group consisting of H, C_1 - C_8 straight-chain alkyl, C_1 - C_8 branched-chain alkyl, C_1 - C_8 straight-chain substituted alkyl, C_1 - C_8 branched-chain substituted alkyl.

5. A compound according to claim 4, wherein

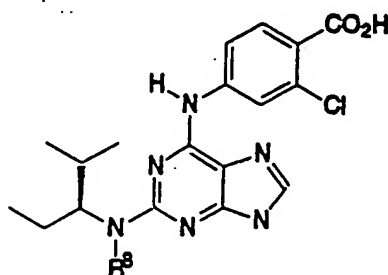
X is COOH; and

R^6 is a member independently selected from the group consisting of H, $-CH_3$, $-(CH_2)_3NHC(=NH)NH_2$, $-CH_2CONH_2$, $-CH_2CO_2H$, $-CH_2SH$, $-(CH_2)_2CONH_2$, $-(CH_2)_2CO_2H$, $-CH_2(4\text{-imidazolyl})$, $-CH(CH_3)CH_2CH_2$, $-CH_2CH(CH_3)_2$, $-(CH_2)_4NH_2$, $-(CH_2)_2SCH_3$, $-CH_2Ph$, $-CH_2OH$, $-CH(CH_3)OH$, $-CH_2(3\text{-indolyl})$, $-CH_2(4\text{-hydroxyphenyl})$ and $-CH(CH_3)_2$.

1 6. A compound according to claim 4, wherein

2 R¹ and R² are independently members selected from the group consisting
3 of H and aryl substituted in at least one of positions 3, 4, 5 with a member independently
4 selected from the group consisting of halogen, alkoxy, trihalomethyl, amino, hydroxyl,
5 thiol, sulfonic acid, sulfonic acid, amide, ester and carboxylic acid.

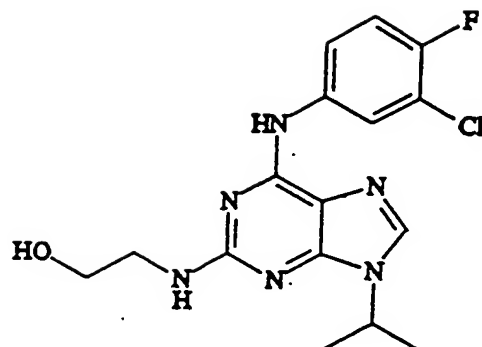
1 7. A compound according to claim 6, having the structure



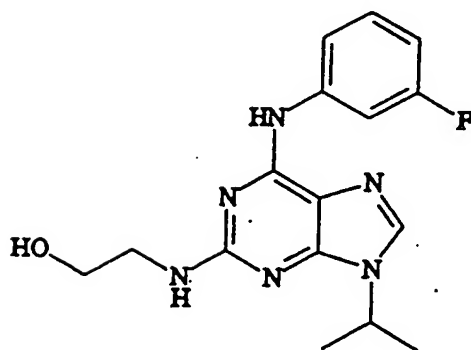
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3 wherein R⁸ is a member selected from the group consisting of H, substituted benzyl
4 groups, substituted C₁-C₃ straight chain alkyl group, heterocycles and substituted
5 heterocycles.

1 8. A compound according to claim 6, wherein said aryl group is a
2 member selected from the group consisting of polycyclic aromatic hydrocarbons and
3 substituted polycyclic aromatic hydrocarbons.

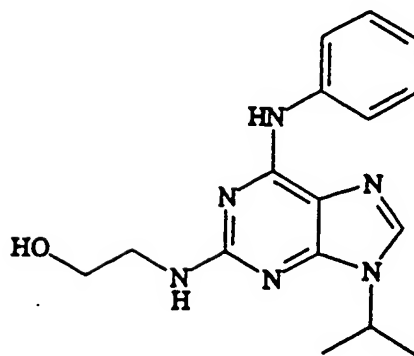
1 9. A compound according to claim 1 having a structure selected from
2 the group consisting of



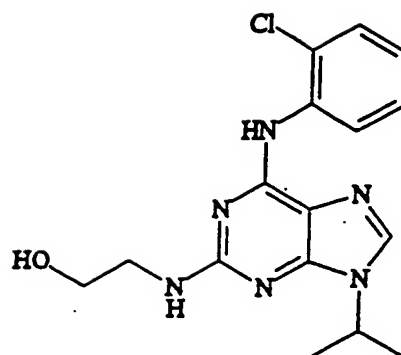
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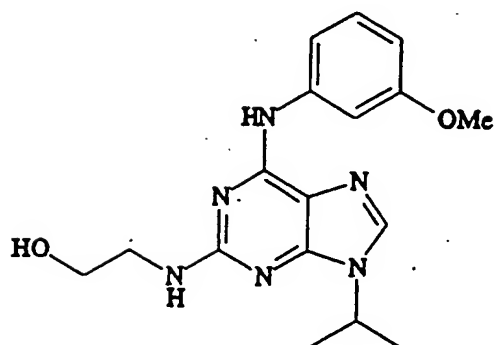
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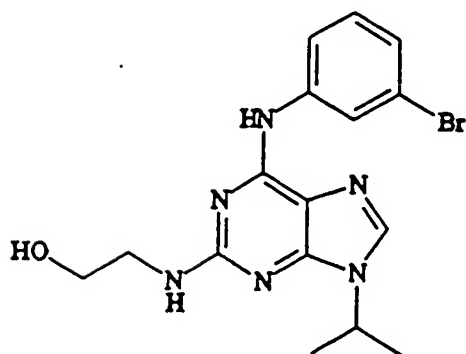
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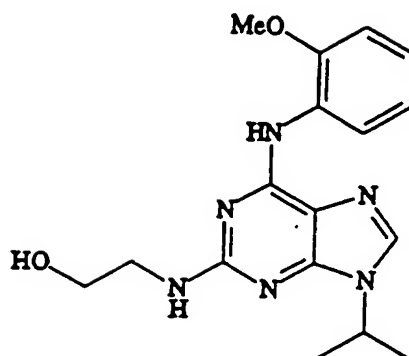
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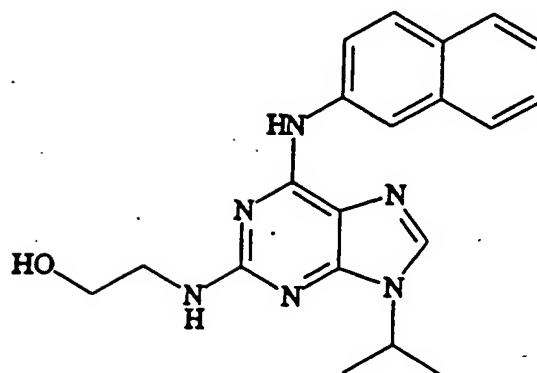
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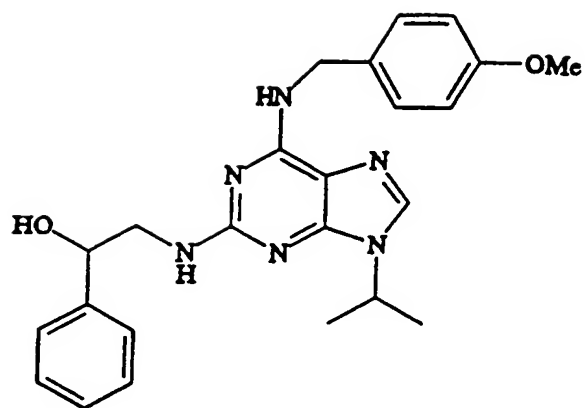
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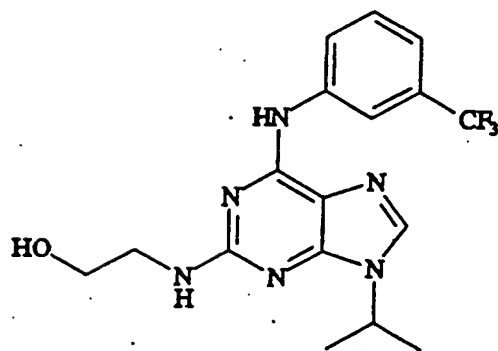
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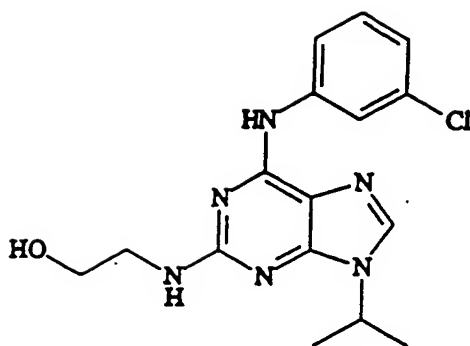
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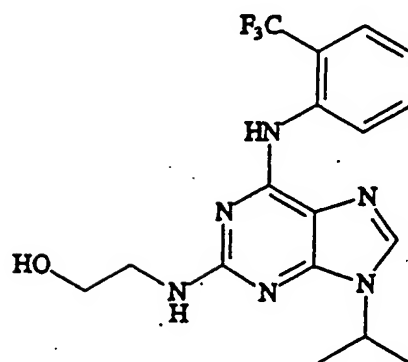
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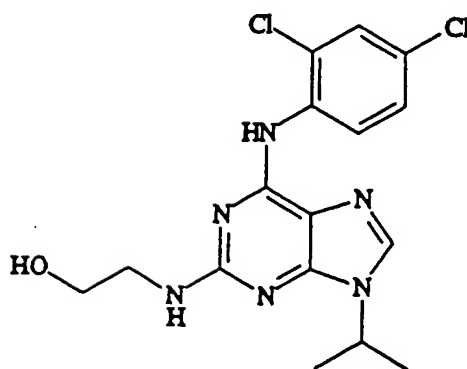
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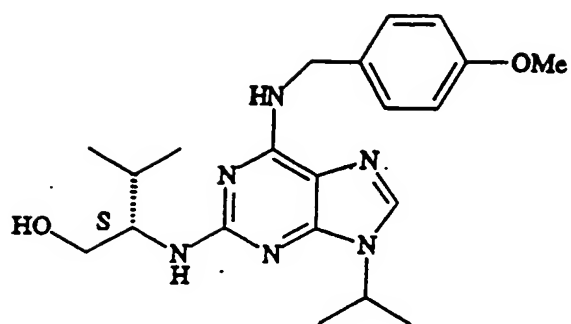
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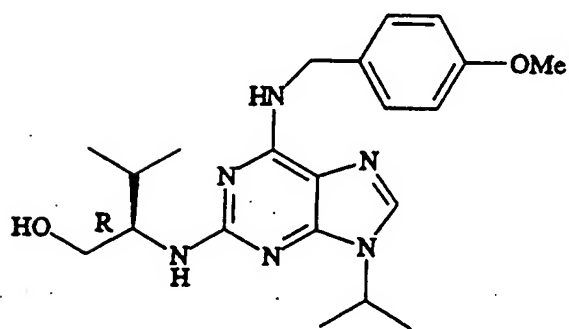
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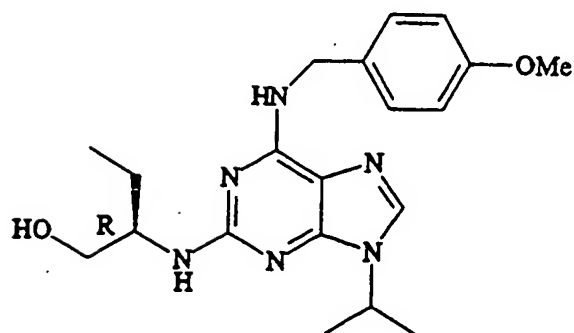
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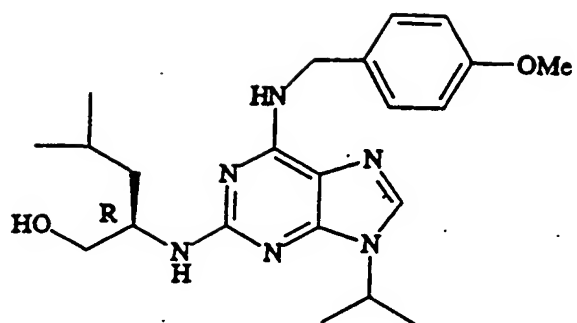
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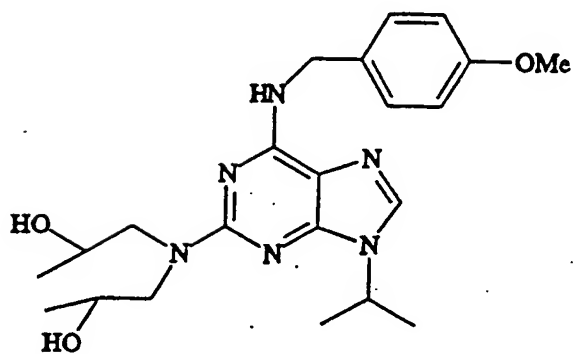
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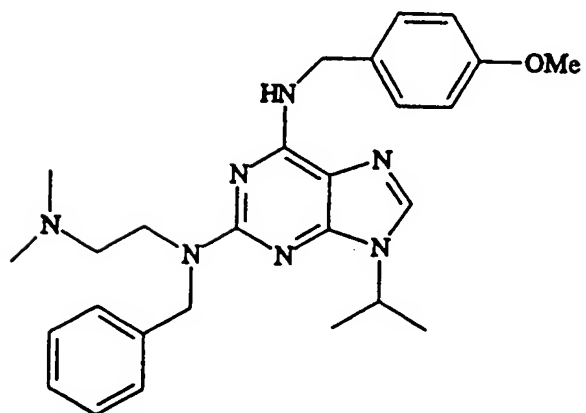
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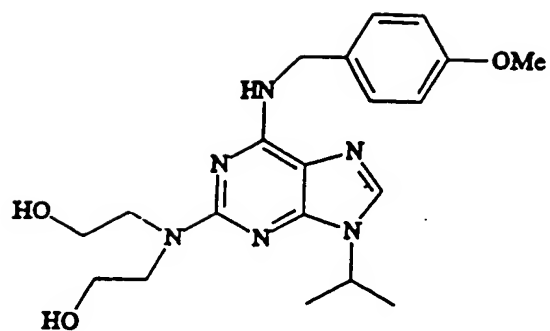
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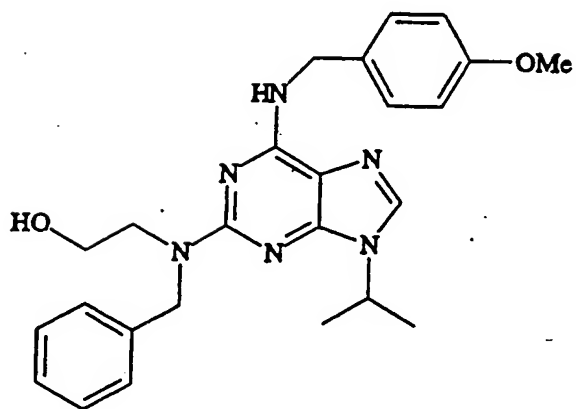


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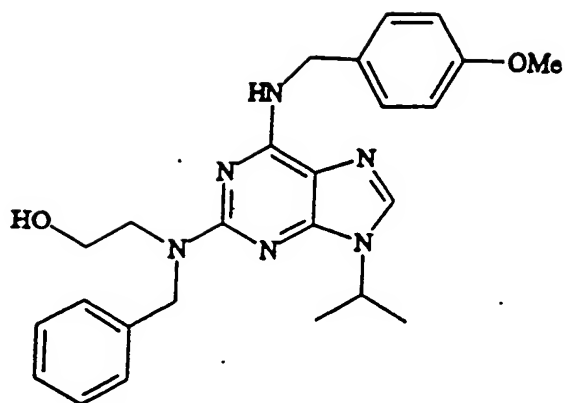


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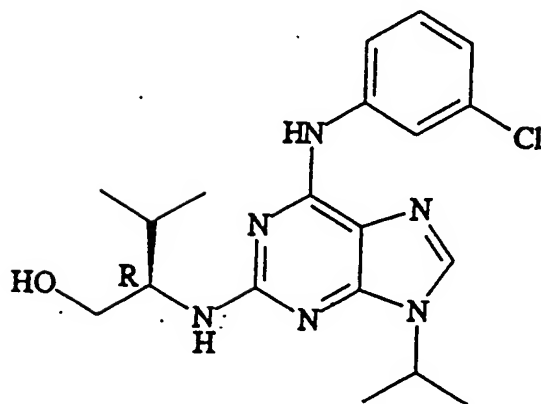
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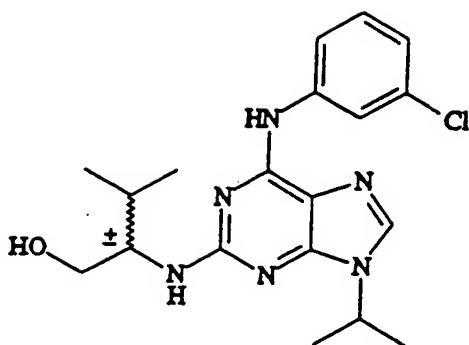
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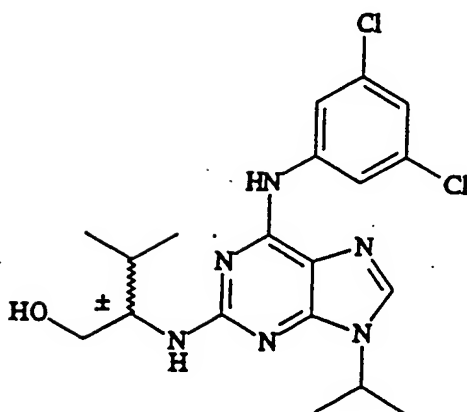
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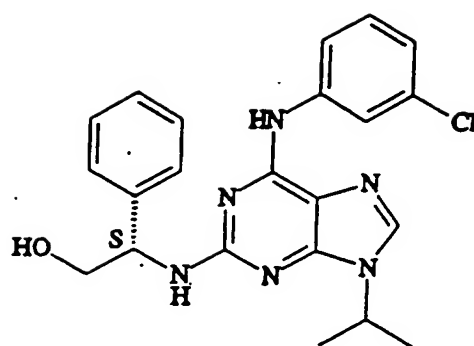
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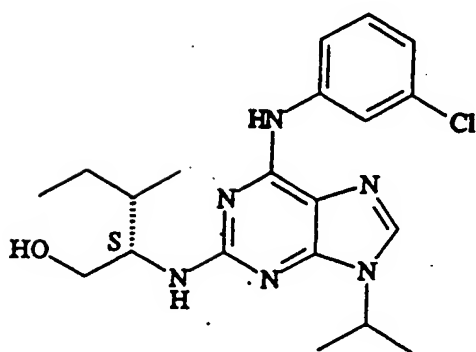
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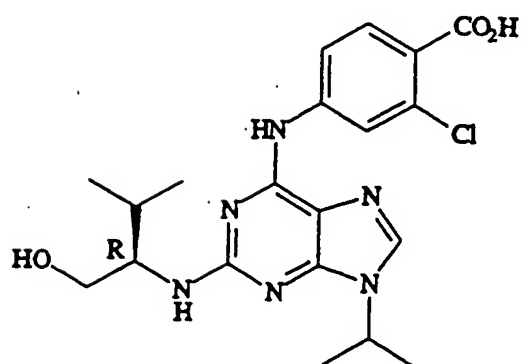
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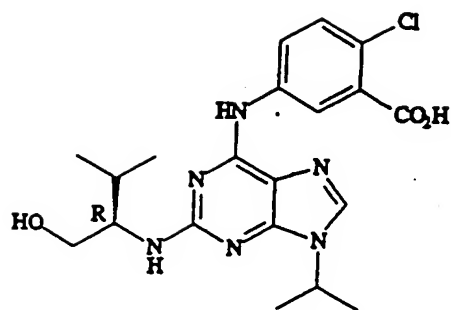


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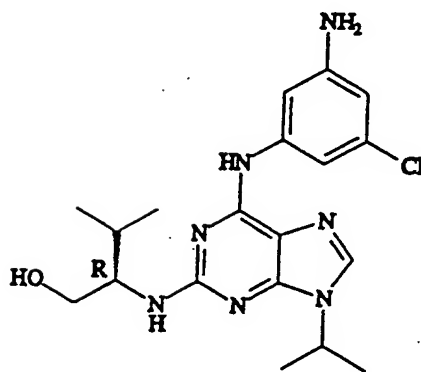


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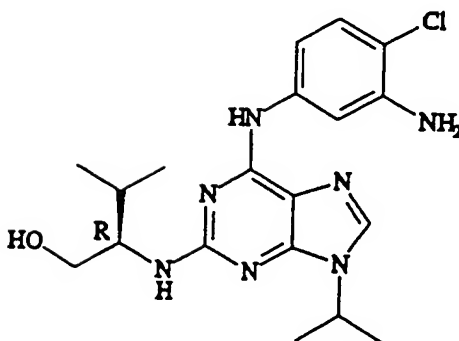
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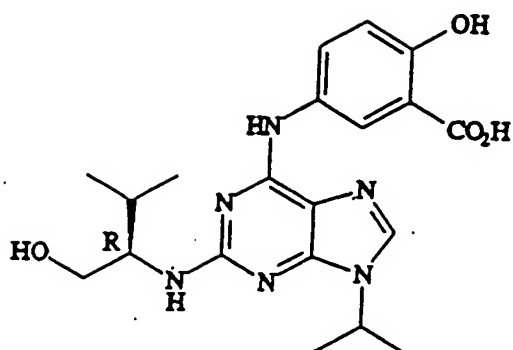


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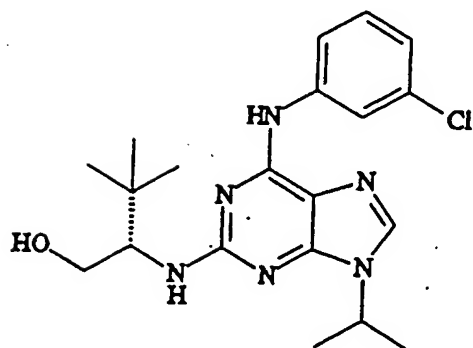


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1 10. A pharmaceutical composition comprising a compound in
2 accordance with claim 1 and a pharmaceutically acceptable carrier.

1 11. A pharmaceutical composition comprising a compound in
2 accordance with claim 9 and a pharmaceutically acceptable carrier.

1 12. A method of inhibiting a protein selected from the group consisting
2 of protein kinases, G proteins and polymerases, said method comprising contacting said
3 protein with a compound of claim 1.

1 13. A method in accordance with claim 12, wherein said protein is a
2 protein kinase.

1 14. A method in accordance with claim 13, wherein said protein kinase
2 is a cyclin-dependent kinase.

1 15. A method in accordance with claim 14, wherein said cyclin-
2 dependent kinase is a member selected from the group consisting of CDK1 (CDC2),
3 CDK2, CDK3, CDK4, CDK5, CDK6, CDK7 and CDK8.

1 16. A method in accordance with claim 15, wherein said protein kinase
2 is a member selected from the group consisting of CDK1 and CDK5.

1 17. A method of treating a cellular proliferative disease, said method
2 comprising administering to a mammal having said disease a therapeutically effective
3 amount of a compound of claim 1.

1 18. A method of inhibiting the growth of a tumor cell, said method
2 comprising contacting said tumor cell with a compound of claim 1.

1 19. A method in accordance with claim 18 wherein said tumor cell is
2 selected from the group consisting of lung, colon, breast, ovarian, prostate and hepatic
3 cells.

1 20. The method in accordance with claim 18 wherein said tumor cell is
2 in a mammalian subject.

1 21. The method in accordance with claim 18 wherein said compound is
2 formulated in a pharmaceutically acceptable form with an excipient or carrier.

1 22. The method in accordance with claim 18 wherein said compound is
2 administered orally.

1

2

23. The method in accordance with claim 18 further comprising the step of observing for a reduction in the growth of said tumor cell.

1

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3

24. A method of inhibiting the growth of a tumor cell in a mammalian subject, said method comprising administering to said subject a therapeutically effective amount of a compound of claim 1.

1

2

3

25. A method of treating a neurodegenerative disease, said method comprising administering to a mammal having said disease a therapeutically effective amount of a compound of claim 1.

1/2

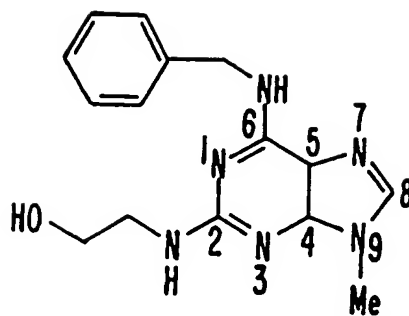


FIG. 1.

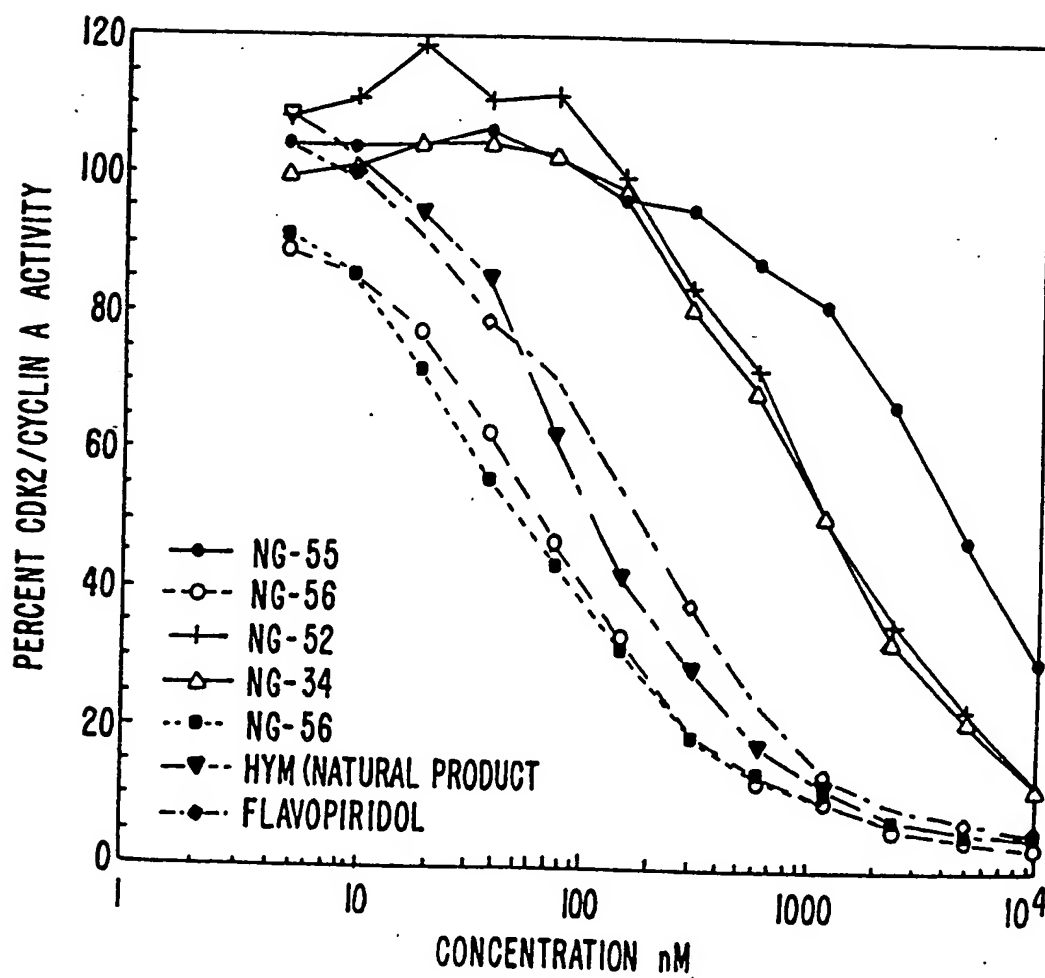


FIG. 2.

2/2

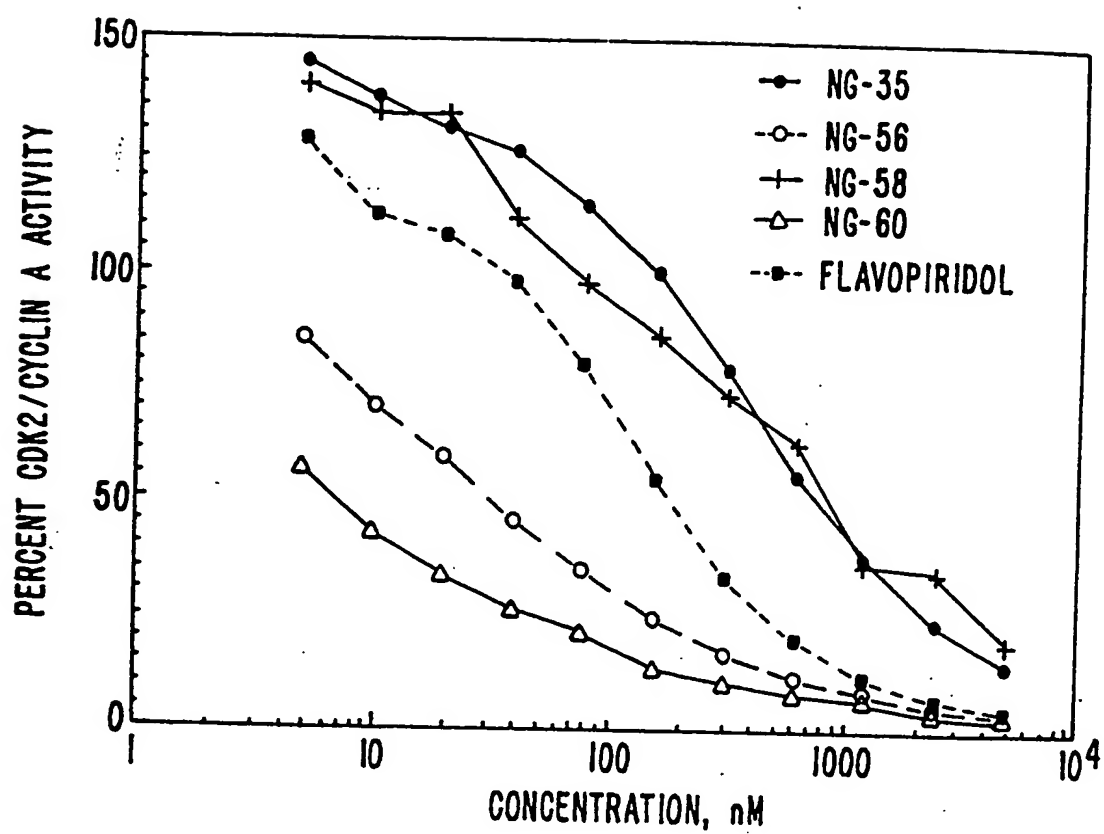


FIG. 3.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/16388

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>VESELY J ET AL: "INHIBITION OF CYCLIN-DEPENDENT KINASES BY PURINE ANALOGUES" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 224, no. 2, 1 September 1994, pages 771-786, XP002009709 cited in the application see page 780; table 2, the compounds no. 53, 55 and 57</p>	1,2,10, 12-25
X	<p>HAVLICEK L ET AL: "Cytokinin-derived cyclin-dependent kinase inhibitors: synthesis and cdc2 inhibitory activity of olomoucine and related compounds" JOURNAL OF MEDICINAL CHEMISTRY, vol. 4, no. 40, 14 February 1997, page 408 408 XP002079219 see page 409; table 1, the compounds no. 22-26</p>	1,2,10, 12-25
X	<p>GRAY N S ET AL: "Combinatorial Synthesis of 2,9-Substituted Purines" TETRAHEDRON LETTERS, vol. 38, no. 7, 17 February 1997, page 1161-1164 XP004034223 see page 1163; the compound no. 11</p>	1,2,10
P,X	<p>SCHOW S R ET AL: "Synthesis and Activity of 2,6,9-Trisubstituted Purines." BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 7, no. 21, 1997, pages 2697-2702, XP002084930 see pages 2700-2710; table 1, the compounds no. 5, 7, 9, 13, 14, 17, 18, 22, 24, 25, 27, 29, 30, 33-36, 38, 44-49, 51, 52 and 54-56</p>	1-6,9-25
P,X	<p>DE AZEVEDO W F ET AL: "Inhibition of cyclin-dependent kinases by purine analogues. Crystal Structure of human cdk2 complexed with roscovitine" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 243, no. 1/2, 1997, pages 518-526, XP002084931 see page 521; table 1, the compounds no. 92, 93-95, 96, 99-103 and 104</p>	1,2,10, 12-25
P,X	<p>WO 98 05335 A (CV THERAPEUTICS INC ;BLUM CHERI LYNN (US); LUM ROBERT T (US); MACK) 12 February 1998 see page 57 - page 58; claim 1</p>	1-25
P,X	<p>WO 98 16528 A (CHIRON CORP ;UNIV CALIFORNIA (US)) 23 April 1998 see page 17; claim 1</p>	1-25

INTERNATIONAL SEARCH REPORT

Int lional Application No
PCT/US 98/16388

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D473/16 C07D487/04 A61K31/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 20842 A (CENTRE NAT RECH SCIENT ;MEIJER LAURENT (FR); BISAGNI EMILE (FR); L) 12 June 1997 see the whole document; in particular pages 34-35, claim 1 ---	1-6, 9-25
X	WO 97 16452 A (CIBA GEIGY AG ;ZIMMERMANN JUERG (CH); CAPRARO HANS GEORG (CH); PET) 9 May 1997 see the whole document; in particular pages 83-84, claim 1 --- -/--	1-6, 9-25

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

20 November 1998

Date of mailing of the international search report

17. 12. 98

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2

Authorized officer

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/16388

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 12-25 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 1,2,10,12-25
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 98/16388

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 1,2,10,12-25

The novelty-search on the compounds of present claim 1 revealed such a vast amount of documents which were novelty-destroying with respect to this claim, that the search report had to be limited (cf. Rule 33.3(b) PCT) to those compounds of present claim 1, wherein:

R1 and R2 are independently members selected from H; optionally substituted aryl and benzyl, which may optionally be substituted on the phenyl portion; R3 represents isopropyl; and wherein R4 and R5 are as defined in present claim 1 (i.e. the search report can be considered as being complete with respect to claims 3-9 and 11 of the present application).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/16388

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9720842	A	12-06-1997	FR 2741881 A EP 0874847 A	06-06-1997 04-11-1998
WO 9716452	A	09-05-1997	AU 7296896 A CA 2234609 A EP 0874846 A	22-05-1997 09-05-1997 04-11-1998
WO 9805335	A	12-02-1998	AU 3900097 A	25-02-1998
WO 9816528	A	23-04-1998	AU 4920397 A	11-05-1998